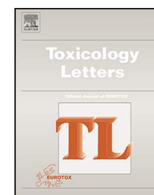
Toxicology Letters 236 (2015) 131–137

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet



Study of urinary 2-{[2-(acetylamino-2-carboxyethyl]sulfanyl} butanedioic acid, a mercapturic acid of rats treated with maleic acid

Yu-Syuan Luo [a,1], Hsin-Yun Tsai [a,1], Hsin-Chang Chen [a], Charlene Wu [a], Li-Ching Shen [b], Wen-Sheng Chung [b], Su-Yin Chiang [c,*], Kuen-Yuh Wu [c,**]

[a] Institute of Occupational Medicine and Industrial Hygiene, College of Public Health, National Taiwan University, Taipei 10055, Taiwan
[b] Department of Applied Chemistry, College of Science, National Chiao Tung University, Hsinchu 30010, Taiwan
[c] School of Chinese Medicine, China Medical University, Taichung 404, Taiwan

HIGHLIGHTS

- MAMA was first synthesized, purified and characterized.
- A LC–MS/MS method was successfully developed for the analysis of MAMA.
- MAMA was present in rat urine after MA exposure and increased with dose.
- Urinary MAMA was validated to serve as a biomarker of current MA exposure in rats.

GRAPHICAL ABSTRACT

ARTICLE INFO

Article history:
Received 18 March 2015
Received in revised form 9 May 2015
Accepted 12 May 2015
Available online 18 May 2015

Keywords:
Maleic acid
Mercapturic acid
Detoxication
LC–MS/MS

ABSTRACT

Maleic anhydride was reported illegally adulterated into starch to prepare traditional foods for decades in Taiwan. Maleic acid (MA), hydrolyzed from maleic anhydride, could cause kidney damages to animals. The potential health effects due to long-term MA exposures through food consumption have been of great concerns. Assessment of the dietary MA exposures could be very difficult and complicated. One of the alternatives is to analyze an MA-specific biomarker to assess the daily total MA intake. Therefore, this paper aimed to study the mercapturic acid of MA, 2-{[2-(acetylamino)-2-carboxyethyl]sulfanyl} butanedioic acid (MAMA), with our newly-developed isotope-dilution online solid-phase extraction liquid chromatography tandem mass spectrometry (ID-SPE-LC-MS/MS) method. MAMA was first synthesized, purified, and characterized with NMR to reveal two diastereomers and used for developing the analytical method. The method was validated to reveal excellent sensitivity with a LOD at 16.3 ng/mL and a LOQ at 20.6 ng/mL and used to analyze MAMA in urine samples collected from Sprague-Dawley rats treated with a single dose of 0 mg/kg, 6 mg/kg, and 60 mg/kg ($n = 5$) of MA through gavage. Our results show dose-dependent increases in urinary MAMA contents, and 70% MAMA was excreted within 12 h with no gender differences ($p > 0.05$). A half life of urinary MAMA was estimated at 6.8 h for rat. The formation of urinary MAMA validates it as a chemically-specific biomarker for current MA exposure. Future study of MA metabolism in vivo will elucidate mechanisms of MAMA formation, and analysis of

Abbreviations: COSY, correlation spectroscopy; DDW, double de-ionized water; GSH, glutathione; HESI, heated electro spray ionization; HMBC, heteronuclear multiple-bond correlation spectroscopy; HMQC, heteronuclear multiple-quantum correlation spectroscopy; LOD, limit of detection; LOQ, limit of quantification; MA, maleic acid; MAMA, 2-{[2-(acetylamino)-2-carboxyethyl]sulfanyl}butanedioic acid; PS1D, pure shift 1D spectroscopy; PBS, phosphate buffered saline.

* Corresponding author. Tel.: +886 4 2205 3366 3305; fax: +886 4 2203 2295.
** Corresponding author at: Rm 721, No 17, Xuzhou Rd, Zhongzheng Dist, Taipei 10055, Taiwan. Tel.: +886 2 3366 8091; fax: +886 2 3366 8267.
E-mail addresses: sychiang@mail.cmu.edu.tw (S.-Y. Chiang), kuenyuhwu@ntu.edu.tw (K.-Y. Wu).
[1] Authors contributed equally to this work.

http://dx.doi.org/10.1016/j.toxlet.2015.05.011
0378-4274/© 2015 Elsevier Ireland Ltd. All rights reserved.

this marker in epidemiology studies could help to shed light on the causal effects of MA on human.
©2015 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Maleic anhydride, an illegal food additive, was intentionally added in starch to prepare a wide variety of traditional foods (e.g., tapioca, rice noodles and oden), in order to ameliorate viscosity, texture, and stability of the foods for decades in Taiwan. Maleic anhydride can be completely hydrolyzed to maleic acid (MA) at room temperature with a relative humidity of 96% (Rosenfel and Murphy, 1967). Harrison and Harrison (1954),Albander et al. (1982), and Eiamong et al. (1995) reported that MA could cause kidney effects to rats and dogs. The symptom is similar to the Fanconi syndrome in human, which the tubular reabsorption is malfunctioned, and glucose, amino acids, phosphate and bicarbonate could not be reabsorbed in proximal tubule (Albander et al., 1982; Harrison and Harrison, 1954). The prevalence rates of end-stage renal disease in Taiwan is ranked first worldwide (USRDS, 2013). Daily intakes of MA have been of great concerns.

The study of the potential adverse effects of MA requires information of MA exposures through consumption of the MA-modified starch foods. The assessment of MA exposures through food consumption could be very difficult and complicated due to variation of daily food consumption rate and MA contents in the foods. One of the alternatives is to analyze a MA-specific biomarker to assess daily total MA exposures. In terms of biological monitoring, urine samples are non-invasive and easy to access. If a urinary biomarker of MA can be detected, it can not only serve as a biomarker to assess total MA exposures, but also imply MA metabolism and/or detoxication in vivo. Although the metabolism of MA is not well understood, glutathione-*S*-transferase (GST) usually plays an important role in detoxification of industrial chemicals to form their corresponding glutathione conjugates, which are further metabolized to *N*-acetyl cysteine adducts, also named as mercapturic acids (Habig et al., 1974; Hecht et al., 2008). These mercapturic acids have been well studied to serve as biomarkers to assess current exposures for these chemicals in our living environments and/or at workplaces (Alary et al., 1995; Eckert et al., 2013; Huang et al., 2007; Kotapati et al., 2014; Kuiper et al., 2010; Maestri et al., 1997; Perbellini et al., 2002). If MA is also detoxified by GST, the resulting MA glutathione conjugates can be further metabolized to 2-{[2-(acetylamino)-2-carboxyethyl]sulfanyl}butanedioic acid, the mercapturic acid of MA (MAMA) excreted through urine. Therefore, the aims of this study were to investigate urinary MAMA by using our newly-developed isotope-dilution online solid-phase extraction liquid chromatography tandem mass spectrometry (ID-SPE-LC-MS/MS) method for rats treated with MA. Results from this study will not only validate urinary MAMA as a biomarker for MA exposure, but also elucidate in vivo metabolism of MA in part.

In order to accurately quantify urinary MAMA, MAMA and [$^{13}C_2$]-MAMA were first synthesized, purified, and characterized to prepare their standard solutions for developing the LC–MS/MS method. After validation of this method, MAMA in urine samples collected from rats treated with single dose of 6 and 60 mg/kg (n = 5) of MA were analyzed, and compared with those of control rats. To the best of our knowledge, MAMA is first quantified in urine of rats treated with MA. Further studies by monitoring urinary MAMA as a biomarker for human exposed to MA will help to shed light on the potential health effects caused by daily intakes of MA through food consumption.

2. Materials and methods

2.1. Caution

MA is irritative and corrosive to eye, skin and lung. Acetonitrile and formic acid should be handled with proper personal protective equipment and operated in a well-ventilated hood.

2.2. Chemicals

MA (99.0%, 500 g) was purchased from Showa (Tokyo, Japan). *N*-acetyl cysteine (NAC) (≧99%, 25 g), phosphate buffered saline (PBS), formic acid (≧98%, 500 mL), and [$^{13}C_2$]-MA were obtained from Sigma–Aldrich (St. Louis, MO). Acetonitrile (≧99.9%, 4 L) was supplied by Merck (Darmstadt, Germany). Double de-ionized water (DDW) was prepared by a Milli-Q system (Billerica, MA).

2.2.1. Synthesis, purification and characterization of MAMA conjugate

MA (0.10 mmol, 11.67 mg) and NAC (0.11 mmol, 18.01 mg) were added into 10 mL of PBS buffer (2 g of PBS tablet dissolved in 200 mL of DDW) and stirred at 37 °C for 96 h. MAMA was purified with an LC–UV system consisted of a semi-preparative C18 column (5 μm, 10 × 250 mm, Phenomenex Co., Torrance, CA), a binary pump (Jasco PU-980) and an UV–vis detector (205 nm, Jasco UV-975, Easten, MD). Isocratic elution with mobile phase A (0.1% FA in H_2O) and B (acetonitrile) at a ratio of 88:12 and a flow rate of 1.2 mL/min was adopted for chromatographic separation. MAMA was collected at retention time between 16.5 and 17.5 min by using a fraction collector (CHF121SA, Toyo Seisakusho Kaisha, Ltd., Japan), and then the collected solutions were combined and dried under vacuum. MAMA was characterized by using a triple quadruple tandem mass spectrometer (MS/MS) with a heated electrospray ionization (HESI) source in negative ion mode. MAMA was prepared in DDW and analyzed through direct infusion at a flow rate of 10 μL/min. A spray voltage was set at 2000 V, and sheath gas and auxiliary gas flow rate were at 35 and 10 psi; capillary and HESI temperature were set at 150 °C and 200 °C, respectively. Product ion spectrum of MAMA was obtained with a collision gas pressure of 1.5 mTorr and a scan period of 1 min. The precursor ion was m/z 278 ($[M-H]^-$) and the product ions were m/z 149 ($[M-C_5H_8NO_3-H]^-$), m/z 162 ($[M-C_4H_5O_4-H]^-$), and m/z 115 ($[M-C_5H_8NO_3S-H]^-$). MAMA was dissolved in deuterium oxide and further characterized with NMR (Varian VNMRS-600 NMR Spectrometer). Results from ^1H and ^{13}C NMR analysis were described as following:

^1H NMR (600 MHz, D_2O).MAMA (Diastereomer A): δ [ppm] = 4.51 (dd, $J_{3,a'}$ = 7.8 Hz, $J_{3,a}$ = 4.8 Hz, 1H, H-3), 3.65–3.68 (m, 1H, H-c), 3.11 (dd, $J_{a,a'}$ = 13.8, $J_{a,3}$ = 4.8 Hz, 1H, H-a), 2.99 (dd, $J_{a',a}$ = 13.8, $J_{a',3}$ = 7.8 Hz, 1H, H-a′), 2.84 (dd, $J_{d,d'}$ = 17.4, $J_{d,c}$ = 9.6 Hz, 1H, H-d), 2.70 (dd, $J_{d',d}$ = 15.0, $J_{d',c}$ = 6.0 Hz, 1H, H-d′), 1.91 (s, 3H, H-1); **MAMA (Diastereomer B):** δ [ppm] = 4.52 (dd, $J_{3,a'}$ = 8.4, $J_{3,a}$ = 4.8 Hz, 1H, H-3), 3.65–3.68 (m, 1H, H-c), 3.13 (dd, $J_{a,a'}$ = 14.4, $J_{a,3}$ = 4.2 Hz, 1H, H-a), 2.91 (dd, $J_{a',a}$ = 13.8, $J_{a',3}$ = 8.4 Hz, 1H, H-a′), 2.84 (dd, $J_{d,d'}$ = 17.4, $J_{d,c}$ = 9.6 Hz, 1H, H-d), 2.68 (dd, $J_{d',d}$ = 17.4, $J_{d',c}$ = 6.0 Hz, 1H, H-d′), 1.92 (s, 3H, H-1).

^{13}C NMR (150 MHz, D_2O).MAMA (A form): δ [ppm] = 178.1 (s, C-b), 177.2 (s, C-e), 176.8 (s, C-2), 176.3 (s, C-4), 55.3 (s, CH-3), 45.1 (s, CH-c), 38.8 (s, CH_2-d), 35.0 (s, CH_2-a), 24.2 (s, CH_3-1); **MAMA (B form):** δ [ppm] = 178.0 (s, C-b), 177.2 (s, C-e), 176.8 (s, C-2), 176.3 (s, C-4), 54.8 (s, CH-3), 44.3 (s, CH-c), 38.6 (s, CH_2-d), 34.6 (s, CH_2-a), 24.2 (s, CH_3-1).

Download English Version:

https://daneshyari.com/en/article/2598687

Download Persian Version:

https://daneshyari.com/article/2598687

Daneshyari.com